Beyond the Biomass
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CHAPTER 23

Holistic approaches to the study of populations, nutrient pools and fluxes: Limits and future research needs

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Holism is a theory which views reality as being made up of 'wholes' that are more important in terms of functioning than the simple sum of their parts. This theory has been extended to various disciplines; in soil science, the holistic approach has been used to investigate nutrient transformations (Stevenson, 1986). Using the techniques which are currently available, it is impossible to monitor each inorganic and organic compound and to quantify the rates of each abiotic and biotic reaction in a complex system such as soil. Thus, the system has been partitioned into pools which have a functional meaning; fluxes among pools represent physical processes (for example, leaching and volatilisation) or abiotic and/or biotic transformations. This has allowed a better mathematical description of the system (Stevenson, 1986).

The major limitations to the discrete pool approach is that the system is likely to vary continously and that the models based on pools will never completely reflect biological, chemical or physical reality. Current techniques allow some pools and fluxes to be evaluated with a reasonable degree of accuracy, whereas this is not the case with other pools and fluxes described by mathematical models. Further insights in nutrient cycling require: a reconciliation of differences among conceptual, mathematical and operational definitions of pools; a more detailed description of systems involving a larger number of pools and fluxes than those currently determined; and a direct estimation of functional pools and fluxes identified in conceptual models.

Nutrient cycling is rather complex from a biological, chemical and physical perspective. In view of the limited space, it will not be possible to discuss here the major research needs of nutrient cycling. The discussion focuses on nutrient mineralisation and immobilisation processes, whose complexity makes them an excellent testing ground for assessing new methods and their application to mathematical modelling.

Particular attention is given to conceptual and experimental procedures for determining new abiotic and biotic pools and respective fluxes with a functional meaning. The current model describing

the mineralisation and immobilisation of nutrients in soil organic matter is based on the following basic principles:

- microbial biomass is a significant source and sink of nutrients
- microbial decomposition of organic matter and microbial synthesis occur simultaneously in soil
- nutrients in the organic matter pool are heterogeneous in terms of biological activity (a fraction cycles very rapidly but some components cycle very slowly)

DEFINITION AND DETERMINATION OF NEW POOLS AND FLUXES IN MINERALISATION AND IMMOBILISATION PROCESSES

Microbial pools

Microbial biomass has been considered as an undifferentiated whole in studies on mineral cycling and energy flow in soil. This approach has been made possible by the availability of the fumigation technique which allows a direct and accurate estimation of relative carbon, nitrogen, phosphorus and sulphur content (Jenkinson and Ladd, 1981; Jenkinson, 1988). Other methods based on the extraction of specific components of living microbial cells, such as the adenosine triphosphate (ATP) method, or on the physiological response of microrganisms to an exogenous substrate, such as the substrate-induced respiration (SIR) method, are as easy to use and not as time-consuming as the $CHCl_3$ fumigation-extraction or fumigation-incubation methods. However, these methods permit only the indirect determination of the nutrient content of microbial biomass (Anderson and Domsch, 1978; Jenkinson, 1988). Thus, the risk of error is higher in indirect than direct methods. New methods for characterising microbial subpools should permit the direct determination of the nutrient content.

The use of molecular biology techniques, such as DNA extraction, purification, cleaving by restriction endonucleases and probing using DNA/DNA hybridisation techniques, has the potential to provide a better definition of the composition and ecology of soil microflora. However, its contribution to a better characterisation of nutrient cycling may be irrelevant. The number of a microbial species is not related to the activity of this species. Many factors, all resulting from the peculiarity of the soil environment, are responsible for the lack of a relationship between microbial numbers and activity (Nannipieri et al., 1990). The extraction and purification procedures in soil molecular biology techniques could be developed for estimating the nutrient content of microbial subpools with a functional role in nutrient cycling. Molecular probing using DNA/DNA hybridisation requires extraction of DNA from soil. This is usually carried out in two ways: microorganisms may be lysed directly in soil prior to extraction; or microbial cells may be separated from soil and lysed to recover DNA (Sayler et al., 1992; Trevors, 1992). The latter technique could be developed and used to obtain discrete microbial subpools such as bacterial, fungal or protozoan fractions with a functional role in nutrient cycling. The separation of cells from soil allows the determination of the nutrient content if the extraction and purification procedures are sufficiently effective. Obviously, the technique should be easy to apply and not time-consuming.

Another possible approach for partitioning microbial biomass in subpools is the discrimination between fungi and bacteria by specific inhibitors. Commonly used antibiotics, such as the bactericide streptomycin and the fungicide cycloheximide, specifically inhibit protein synthesis acting on bacterial (70s) and fungal (80s) ribosomes, respectively (Lukens, 1971; Kucers and Bennet, 1979;

Landi et al., 1993). The selective inhibition of the respiratory responses of bacteria and fungi to glucose has been used to determine the bacteria/fungi ratio in soil (Anderson and Domsch, 1973). No conclusive results were obtained when both inhibitors were used singly or in combination to separate the contribution of fungi and bactera to soil respiration and net nitrogen mineralisation rates (Landi et al., 1993). The efficacy of an inhibitor in soil depends upon biotic and abiotic processes (*see* Table 23.1). Interactions between the antibiotic molecule and inorganic colloids or its association with biologically resistant humic substrates may render the inhibitor inaccessible to either decomposer cells or target microorganisms. An antibiotic may be inactivated or used as a carbon and nitrogen source by microorganisms in the soil. Usually, long periods of biocide residence in soil result in microbial community changes and allow indirect or non-target effects to occur (Ingham and Coleman, 1984).

Table 23.1 Some problems in the use of specific microbial inhibitors, such as cycloheximide and streptomycin, to discriminate between the contribution of fungi and bacteria in the soil

1.	Target and non-target effects on soil microflora
2.	The sensitive/insensitive ratio component of soil microflora may vary according to soil type
3.	Microbial inactivitation of the inhibitor
4.	Use of the inhibitor as a source of carbon and nitrogen by soil microorganisms
5.	Adsorption of the inhibitor by soil colloids

The determination of specific biomarkers may be used to discriminate narrower classes of soil microorganisms; for example, ergosterol exists only in fungi, muramic acid has been found in bacteria, and lipopolysaccharides (LPS) are specific for Gram-negative bacteria (Tunlid and White, 1992).

It has been hypothesised that a better characterisation of microorganisms in nutrient cycling may be obtained by discriminating between the active and inactive pools. Turnover times estimated by modellers for microbial biomass range from 1 to 3 years, while values for non-living organic pools ranged from 25 to thousands of years (Parton et al., 1987). In short-term studies, the partitioning of microbial biomass into subpools characterised by different turnover times may make an important contribution to a better knowledge of nutrient cycling. In long-term studies, however, this partitioning may be less feasible and rewarding than the subdivision of the non-living organic pool.

In essence, the partioning of microbial biomass into subpools is required if we are to improve our understanding of nutrient cycling in soil. The use of antibiotics does not seem to solve the problem, especially with long periods of biocide residence in soil. The approach based on the extraction of microbial groups from soil, followed by the determination of the relative nutrient content, seems promising but an appropriate technique has to be devised.

Non-living organic pools

Current models describe soil organic matter quality and nutrient availability in terms of discrete organic matter fractions, or pools, which vary in decomposition activity. Conceptually useful models should include, in addition to microbial biomass, at least pools for non-living but available or active nutrients and stabilised or recalcitrant forms of nutrients. Indeed, isotopic techniques have shown that

a fraction of soil organic matter and immobilised nutrients can be remineralised very rapidly (Duxbury et al., 1989), while ^{14}C dating has shown that some soil organic carbon, and presumably also the nutrients associated with it, have a turnover time of 1000 years or more (Campbell et al., 1967). Paul and Juma (1981) fractionated organic nitrogen into microbial biomass (24-week relative half-life), active non-microbial biomass (77-week half-life), stabilised organic matter (27-year half-life) and old organic matter (600-year half-life). Biomass nitrogen was determined directly by the fumigation-incubation method. Total active nitrogen was determined from total nitrogen and ^{15}N mineralised during laboratory incubation, on the assumption that recently immobilised ^{15}N had uniformily mixed with the active nitrogen pool but entered no other fractions. An old carbon fraction was estimated from ^{14}C dating and a C/N ratio for this fraction was assumed; the stabilised nitrogen was that which remained. Although several assumptions on which these calculations were based are questionable, this remains one of the few experimental determinations of conceptually meaningful organic nitrogen fractions.

Generally, incubations under controlled physical conditions are used to estimate active pools of any nutrient; the size of these fractions does not define an absolute amount of a specific class molecule but instead provides a relative index of biological nutrient availability under a particular set of conditions. The aerobic nitrogen mineralisation procedure described by Stanford and Smith (1972) is the most commonly used measurement of an active nutrient fraction.

Organic matter mineralisation can be studied by considering the elemental bond classes of nutrients; five classes of chemical bonds (C-C, N-C, S-C, S-O-C and P-O-C) were distinguished by Hunt et al. (1986). The phosphorus bonded to carbon was neglected because it was much smaller than the phosphorus linked to oxygen. The sulphur bonded to carbon was considered relatively inert in terms of the microbial attack. Nitrogen was considered to be stabilised due to the bonding with carbon and probably mineralised by organisms oxidising carbon to provide energy; in contrast, sulphur and phosphorus atoms present as esters were considered to be mineralised by the action of extracellular hydrolases according to the need of the element (McGill and Cole, 1981). In the simulation model, based on the concepts developed by Hunt et al. (1986), the activity of sulphate in the soil solution was simulated fairly well, considering the action of sulphohydrolases and the repressive effect of high soil solution sulphate concentration on enyzme production.

Modellers use different terms to name the discrete pools of organic matter. For example, Duxbury et al. (1989) proposed three pools of soil organic matter: labile organic matter, colloidally and chemically protected organic matter, and physically protected organic matter (*see* Table 23.2). The terminology is not well defined because the physical and chemical significance of the various organic fractions, as well as their functioning, is rather vague. It is logical that physico-chemical interactions and protection, such as chemical structures, would also be continuously, rather than discretely, variable. The very old organic fraction may be protected by chemical interactions with mineral colloids; some authors call this 'chemical protection', while others see it as 'physical protection'. Generally, physical protection is considered for the fraction of intermediate age rather than for the very old fraction. The conceptual or experimental basis for this distinction is not clear. The chemical structure of organic molecules, in itself, is insufficient to account for the extreme variation in age and turnover time. Although humic molecules are more resistant to microbial degradation than biopolymers, their intrinsic chemical recalcitrance is much smaller than the observed stabilisation in soil (Stevenson et al., 1989). The half-life of 1000-year-old humic fractions is in the order of weeks when extracted and fractionated fractions are added to unextracted soil.

A better understanding of the stability of soil organic matter to mineralisation will be obtained by improving the knowledge of the chemical structure and the synthesis of extracellular organic nutrient molecules in soil. Current information on organic nitrogen in soil comes from the acidic hydrolysing

Table 23.2 Pools of soil organic matter and nutrients under temperate conditions

Pool	Turnover time (years)	Pool size controls
Microbial biomass	2.5	Substrate availability
Labile organic matter	20.0	Residue inputs, climate
Colloidally and chemically protected organic matter	1000.0	Soil mineralogy, texture
Physically protected organic matter distribution	Depends upon physical disturbances	Tillage and aggregate disruption, soil particle size

Source: Adapted from Duxbury et al. (1989)

procedure which gives amino acids and amino sugars as the main identifiable organic nitrogen compounds in the soil hydrolysate (Stevenson, 1982). The soluble nitrogen not accounted for as NH_3 or known compounds is the hydrolysable unknown fraction (10-20% of soil nitrogen); 20-35% of the total is not solubilised by acid hydrolysis and is termed 'acid insoluble nitrogen', the chemical structure of which is unknown. It is almost impossible to formulate plausible hypotheses on the presence and experimental procedures for determining organic nitrogen pools if the chemical structure of most of the organic nitrogen in soil is not known. Such knowledge is important but not conclusive for understanding the stability of organic nitrogen to mineralisation; we need to understand how the adsorption of organic nitrogen molecules by soil colloids affects the degradation of organic nitrogen.

Mineralisation and immobilisation processes

Isotope dilution techniques have been used to estimate the gross rates of mineralisation and immobilisation processes. This has involved, for example, adding $^{15}NH_4^+$ and observing the rate at which the atom % ^{15}N enrichment of the NH_4^+ pool declines as a result of mineralisation of organic nitrogen. Consumption of NH_4^+ does not change the ^{15}N enrichment of the pool; thus, it is possible to calculate the gross mineralisation rate from the rate of dilution of pool enrichments. On the other hand, gross rates of NH_4^+ consumption (microbial assimilation and any other consumptive process) are calculated from the disappearance of the ^{15}N label. According to Kirkham and Bartholomew (1954), these calculations can be carried out if three assumptions are valid: microorganisms do not discriminate between ^{14}N and ^{15}N; considered rates of nitrogen processess remain constant during the incubation period; and ^{15}N assimilated during the incubation period is not remineralised. It is well established that, in some processes, discrimination between ^{14}N and ^{15}N occurs (Delwiche and Steyn, 1970) but it is probably not important for transformations of enriched samples over a few days. The error caused by assuming rate is overshadowed by the experimental error with the exclusion of some situations such as when dry soil is re-wetted for incubation or when the soil has been fumigated prior to incubation (Bjarnason, 1988). Remineralisation of immobilised ^{15}N occurs after 1 week (Bristow et al., 1987; Bjarnason, 1988) and thus both the first and third assumptions are valid within the first few days of incubation.

In the holistic approach, mineralisation and immobilisation are considered as single-step processes. Taking nitrogen mineralisation as an example, many metabolic steps, each catalysed by a specific enzyme, are involved in the conversion of organic nitrogen to NH_4^+-N; in the case of proteins, proteases and peptidases are involved in the release of amino acids, and eventually amino acid dehydrogenases and amino acid oxidases catalyse the conversion of amino acids to ammonia (Ladd and Jackson, 1982; Nannipieri et al., 1990). An interesting task would be to determine the critical rate-limiting step of the entire reaction sequence; the kinetics of the overall process could then be defined by those of the rate-limiting reaction (Nannipieri et al., 1990). No marked differences may exist between pure culture and soil in terms of the rate of intracellular metabolic sequences. The rates of extracellular reactions (for example, proteases and peptidases) may be markedly different in soil compared with those of the same reactions in solution; a substrate, rapidly transformed in solution by an enzyme, may be adsorbed in soil by colloids, becoming less accessible to the enzyme. Thus, the reaction rate is retarded and the metabolic step may become the critical rate-limiting step of the overall reaction sequence in soil.

The use of a specific enzyme inhibitor could be used to stop biotic reactions in soil. In this way, it may be possible to block the nitrogen immobilisation so as to permit the determination of the gross nitrogen mineralisation rates. In most bacteria, cellular nitrogen for the synthesis of nitrogen molecules is derived from the amido glutamine group, the amino glutamate group or the amido asparagine group (Reitzer and Magasanik, 1987). When the ammonium ion concentration of the growth medium is greater than 1 mM, ammonia is incorporated directly into glutamate, glutamine and asparagine. When ammonium ion concentration is lower than 0.1 mM, ammonia is incorporated into glutamine only according to the reaction catalysed by glutamine synthetase (GS):

$$NH_3 + \text{glutamate} + ATP \xrightarrow[Mg^{2+}]{} \text{glutamine} + ADP + Pi$$

This occurs because the K_m of glutamine synthetase is lower than the K_m of glutamate dehydrogenase (GHD) which catalyses the reductive amination of 2-oxoglutarate with the formation of glutamate according to the following reaction:

$$NH_3 + \text{2-ketoglutarate} + NADPH \longrightarrow \text{glutamate} + NADP^+$$

Methionine sulphoximine (MSX), a specific inhibitor of GS activity, reduced the ammonia incorporation by 24% and 13% in the O2 and O3 subhorizons, respectively, of a forest soil (Schimel and Firestone, 1989); about 20% of ammonia incorporation occurred through abiotic processes probably involving reactions of NH_3 with activated phenols or quinones. The incorporation was studied in soil slurries, which permit better homogeneous conditions than aerated soils, but these conditions differ from those occurring *in situ*. The inhibitor depressed urease production when 5 g of oven-dried soil were treated with 1.5 ml water containing 1000 μm glucose-carbon and 0 or 250 μm of nitrogen as $(NH_4)_2SO_4$ or KNO_3 (McCarty et al., 1992). We have used the inhibitor in soils incubated at 50% of the water-holding capacity to inhibit the NH_4^+ assimilation and thus separate nitrogen mineralisation from nitrogen immobilisation (Landi et al., 1994). An increase in ammonia concentration occurred in the presence of methionine sulphoximine. Further experiments are needed to ascertain the degree of inhibition of ammonia assimilation by methionine sulphoximine. These experiments should be short term so as to minimise problems related to microbial degradation of the inhibitor.

There is a need to find an accurate, less time-consuming method for determining gross mineralisation and immobilisation rates. The use of specific enzyme inhibitors, blocking one of the two processes, seems promising. However, further research is needed to ascertain the validity of this approach.

CONCLUSION

A more detailed description of nutrient cycling involving a larger number of pools and fluxes than those currently determined is required, particularly for the non-living organic fraction. Analytical methods should be available for determining functional pools and fluxes identified in conceptual models.

There is an urgent need to link chemical and biological studies. Chemists tend to fractionate and study components of soil organic matter without regard to the biological significance of the fractions. Biologists and ecologists have developed concepts and models without fully considering the chemical and physical aspects of pools of different biological stability. It is also necessary to relate studies on mechanisms and processes at the microenvironment level to the possible implications in nutrient cycling, and to improve our knowledge of the chemical nature of associations among organic molecules and between organic and inorganic constituents. However, these studies should not be limited to the chemical aspects; they must also take into account the effects of different types of associations on the stability of soil organic matter components during biological decomposition. Eventually, studies need to be done to determine how the overall architecture of soil affects decomposition processes.

In this review we have emphasised the role of microorganisms as a source and a sink of nutrients. However, we do not want to neglect the importance of acquiring a better knowledge of microbial physiology *in situ*. For example, in order to understand the effect of climatic conditions on nutrient cycling we need to know more about how microorganisms respond physiologically to changes in temperature and moisture. Although there is an extensive bibliography on single-factor response studies (for example, the effect of temperature or of soil moisture on various processes), there is still confusion as to how to integrate these studies in order to predict their quantitative effects on nutrient cycling (Duxbury et al., 1989). Microbial uptake systems are well characterised in pure culture but their significance in natural systems has rarely been considered. In the case of nitrogen, it is generally accepted that microorganisms prefer NH_4^+ and that the decay of organic nitrogen always results in nitrogen mineralisation and the release of NH_4^+ into the inorganic pool. It seems that substrate-specific populations incorporate low-molecular-weight organic nitrogen compounds released from the breakdown of organic matter, and directly assimilate the amino groups (Hadas et al., 1992). The quantitative contribution of this process to nitrogen immobilisation in soil needs to be ascertained.

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